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Chromatin Proteins Do Double Duty

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The histone acetyltransferase MOF (males-absent-on-the-first) is required for the regulation of X chromosome gene dosage compensation in *Drosophila* males. In this issue, Kind et al. (2008) show that MOF is also found on autosomes and that it has two modes of binding: one in males for X chromosome dosage compensation and the other in both sexes for X chromosome and autosomal gene regulation independent of dosage compensation.

The information encoded in genomes is interpreted by proteins that associate with specific chromosomal locations. In dissecting the function of these chromosome-associated proteins, a common assumption has been that a given protein performs a single function in the genome. However, there is a growing realization that a single protein can often be directed to separate regions of the genome by independent mechanisms to perform distinct biological functions. In this issue of *Cell*, Kind et al. (2008) uncover an example of just such a system in the fruit fly *Drosophila melanogaster* by examining the genomic binding distribution of several proteins, including the histone acetyltransferase MOF (males-absent-on-the-first), that are important for X chromosome gene dosage compensation.

MOF, an enzyme that acetylates histone H4 at lysine 16 (H4K16), is a component of the *Drosophila* dosage compensation complex. This complex also includes the *m*ale-specific *l*ethal proteins 1–3 (MSL1, MSL2, MSL3), the *m*ale*l*ess (MLE) protein, and two noncoding RNAs called *roX1* and *roX2* (Straub and Becker, 2007). This complex binds to the single X chromosome of male flies, increasing expression of many X-linked transcripts 2-fold such that expression is equivalent to that in female flies, which have two X chromosomes. Although the detailed mechanism of how the dosage compensation complex acts on the male X chromosome is not clear, it has been proposed that it may promote more efficient transcriptional elongation, possibly by boosting acetylation of H4K16. Indeed, H4K16 acetylation has been shown to "loosen" the conformation of chromatin in vitro (Shogren-Knaak et al., 2006).

Previous studies have found that MSL1 and MSL3 are associated with the transcribed regions of genes, with a binding pattern that is skewed toward the 3′ ends of genes. Therefore, it was perhaps not surprising that Kind et al. (2008) observed that MOF, like the MSL proteins, was also enriched at the 3′ ends of X-linked genes in a dosage compensation-dependent manner. More surprising, however, was their observation of a second mode of MOF binding that was completely independent of dosage compensation, and was found at gene promoters on autosomes and the X chromosome. Previously, MOF had been singled out as an especially interesting member of the dosage compensation complex because it was shown to associate with several components of the nuclear pore (Mendjan et al., 2006). This finding raised the intriguing possibility that MOF performs functions other than dosage compensation at other chromosomal locations. Kind and colleagues now show that MOF does indeed have two functions: a general role in both sexes at the gene promoters located on either autosomes or the X chromosome, and a male-specific role at the 3′ regions of transcribed X-linked genes (Figure 1).

The authors performed several experiments in fly tissue culture cells to build their case that MOF has two different functions. In addition to mapping MOF localization, they determined the binding sites of MSL1 and MSL3, as well as the locations of H4K16 acetylated nucleosomes using ChIP-chip. The analysis of male cells containing a single X

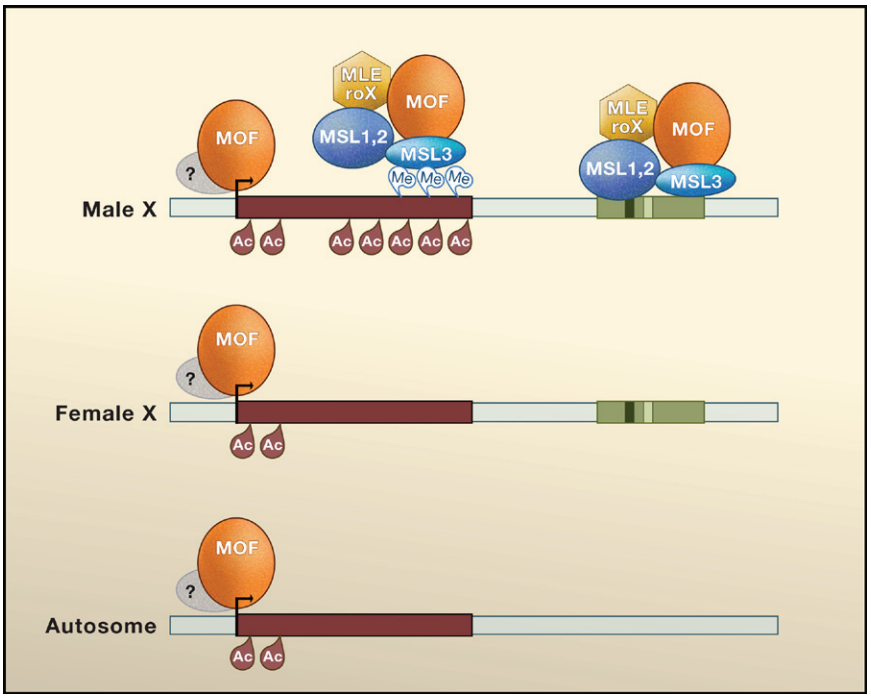

Figure 1. Binding and Functions of the MOF Protein

The dosage compensation complex (DCC) of the fruit fly *Drosophila melanogaster* is recruited to the male X chromosome through DNA sequence-based elements (small bars within green box) recognized by the male-specific lethal proteins 1 (MSL1) and 2 (MSL2). The DCC spreads from these sites to target genes (red box, transcription start and direction indicated by arrow). Here, the DCC associates with the 3′ ends of transcribed regions via MSL3, which binds to trimethylated lysine 36 on histone H3 (H3K36; Me). MOF acetylates histone H4 lysine 16 (H4K16) at these 3′ regions (Ac). In females, MSL2 is not present, so the DCC cannot bind to the recruitment elements and does not spread to the 3′ region of genes. On all chromosomes in both female and male flies, MOF binds to the promoter regions and acetylates H4K16, presumably through interactions with an unknown protein or chromatin modification (gray oval).

chromosome and female cells containing two X chromosomes allowed the authors to identify both dosage compensation-dependent and dosage compensation-independent patterns of protein association and chromatin modification. The authors also examined changes in gene expression in cells depleted of MSL1, MSL3, or MOF using RNA interference. Although the depletion of MSL1 primarily caused misregulation of X-linked genes, depletion of MSL3 and MOF also affected some autosomal genes, suggesting that these proteins do not function only in X chromosome dosage compensation. Further evidence for MOF's autosomal function is based on the 5′ bias of the H4K16 acetylation pattern within transcribed portions of autosomal genes. This acetylation pattern also correlated with the presence of MOF. Finally, depletion of MOF resulted in the loss of most H4K16 acetylation, particularly at the promoters of autosomal genes, suggesting that MOF is responsible for H4K16 acetylation at autosomal gene promoters. The function of MOF and H4K16 acetylation at promoters is not yet known. Studies in the budding yeast *Saccharomyces cerevisiae* do not shed much light on the function of H4K16 acetylation because, in yeast, H4K16 acetylation is transcription independent and is absent from promoters (Liu et al., 2005).

Proteins required for both X chromosome dosage compensation and general chromosome function have been identified in other systems. One prominent example is a condensin subunit in the worm *Caenorhabditis elegans* called mitosis-and-X-associated (MIX-1). This protein associates with all chromosomes during mitosis but also is required for dosage compensation and is associated only with the X chromosome during interphase (Lieb et al., 1998). The function of MIX-1 seems to be specified by its binding partners: the protein DPY-27 in the dosage compensation complex for repression of X-linked genes and a standard SMC4 partner for mitotic chromosome segregation. A similar mechanism for specifying distinct functions could underlie the specification of MOF function.

Targeting of the components of the dosage compensation complex, including MOF, to the *Drosophila* male X chromosome is thought to occur stepwise, with initial recruitment mediated by the MSL1 and MSL2 proteins. This protein pair recognizes a limited number of sites on the X chromosome (Lyman et al., 1997), which may contain short and degenerate DNA sequence motifs that help to distinguish the X chromosome from autosomes (Gilfillan et al., 2007). MOF may make its way to these sites of initial binding through interactions with MSL1 and MSL3 (Straub and Becker, 2007). After this initial recruitment, the dosage compensation complex must spread locally to its target genes. Here, in addition to DNA sequence-based cues, there is also a requirement for the methylation of histone H3 at lysine residue 36 (H3K36) within transcribed regions with a bias to the 3′ ends of genes (Larschan et al., 2007). MSL3 binds to trimethylated H3K36 and thus may recruit MOF to the 3′ regions of the genes. Although this provides an explanation of MOF binding at the 3′ regions of genes, it does not explain how MOF is directed to promoters. The new observation by Kind and coworkers of MOF binding to promoters does not depend on dosage compensation or any of its components. This not only suggests an additional general function for MOF in the regulation of transcription, but also hints at an additional targeting mechanism to get MOF to promoters.

Clues to how MOF is directed to promoters may be provided by the *S. cerevisiae* protein Eaf3, a homolog of MSL3 that is associated with both the promoters and transcribed regions of genes. Eaf3 is a member of both the NuA4 histone acetyltransferase complex and an Rpd3 histone deacetylase complex (Carrozza et al., 2005; Eisen et al., 2001). Like MSL3, Eaf3 binds to methylated H3K36 and directs the Rpd3 complex to the transcribed region of genes. However, Eaf3 is also localized to gene promoters as part of the NuA4 through the targeting action of sequence-specific DNA-bind-

ing transcription factors. It is possible that a similar strategy of targeting MOF to promoters with specific transcription factors or transcription machinery is present in the fly.

Several important questions with general implications are brought to light by the Kind et al. study. What are the apparently independent mechanisms of MOF targeting to the 5′ and 3′ ends of genes? Given that MOF acetylates H4K16 at both sites, what is the molecular function of H4K16 acetylation at these two regions? MOF is undoubtedly representative of many enzymes and chromatin-associated proteins that perform different functions through regulated targeting. The answers to the questions raised by the new work should help to elucidate general mechanisms for how specificity in targeting allows the same protein to perform distinct and independent regulatory functions.

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Follow the Monomer

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Capping proteins limit actin filament growth, but paradoxically increase actin-based cell motility. This has been attributed to funneling of actin monomers to the filament ends that remain uncapped. Using a reconstituted motility system, Akin and Mullins (2008) now demonstrate that filament capping increases Arp2/3-based nucleation and branching, rather than elevating the rate of filament elongation.

Although actin can polymerize into filaments on its own, cells use an army of proteins to control the starting and stopping of this reaction, as well as to organize the filaments into useful structures. Studying the function and regulation of these actin-modulating proteins has been the work of many labs for several decades. The control of actin dynamics is not a mere academic curiosity, but plays a key role in physiological processes such as morphogenesis and immune system function as well as in diseases such as metastatic cancer.

Two important factors for controlling actin dynamics are the Arp2/3 complex, which nucleates new filaments and concomitantly anchors them to the sides of existing filaments, and the capping protein, which binds to the rapidly growing barbed ends of filaments and terminates their growth (reviewed in Pollard and Borisy, 2003). Although these factors have been extensively studied in vitro, the relationship between their biochemical activities and their effects on motility is complex. This is particularly true of capping proteins that block filament growth yet enhance cell motility in vitro and in vivo (van der Gucht et al., 2005; Hug et al., 1995). One explanation for this paradoxical set of observations is the "actin funneling hypothesis," which posits that capping proteins enhance motility by capping most actin filaments in the reaction and funneling the increased number of free actin monomers onto a small subset of filaments that grow with higher rates of elongation (Carlier and Pantaloni, 1997) (Figure 1A).

In their new study, Akin and Mullins (2008) re-examine this question and come to a strikingly different conclusion about the role of capping protein in enhancing motility. They used an established in vitro motility system (Loisel et al., 1999) comprising polystyrene beads coated with the Arp2/3-activator ActA. These beads were incubated in a precise mixture of purified protein components including nonmuscle actin, the